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Characterization of Multiple-Antimicrobial-Resistant *Escherichia coli* Isolates from Diseased Chickens and Swine in China

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Escherichia coli **isolates from diseased piglets (*n* = 89) and chickens (*n* = 71) in China were characterized for O serogroups, virulence genes, antimicrobial susceptibility, class 1 integrons, and mechanisms of fluoroquinolone resistance. O78 was the most common serogroup identified (63%) among the chicken *E. coli* isolates. Most isolates were PCR positive for the increased serum survival gene (*iss*; 97%) and the temperature-sensitive hemagglutinin gene (*tsh*; 93%). The O serogroups of swine *E. coli* were not those typically associated with pathogenic strains, nor did they posses common characteristic virulence factors. Twenty-three serogroups were identified among the swine isolates; however, 38% were O nontypeable. Overall, isolates displayed resistance to nalidixic acid (100%), tetracycline (98%), sulfamethoxazole (84%), ampicillin (79%), streptomycin (77%), and trimethoprim-sulfamethoxazole (76%). Among the fluoroquinolones, resistance ranged between 64% to levofloxacin, 79% to ciprofloxacin, and 95% to difloxacin. DNA sequencing of *gyrA*, *gyrB*, *parC*, and *parE* quinolone resistance-determining regions of 39 nalidixic acid-resistant *E. coli* isolates revealed that a single *gyrA* mutation was found in all of the isolates; mutations in *parC* together with double *gyrA* mutations conferred high-level resistance to fluoroquinolones (ciprofloxacin MIC, ≥8 μg/ml). Class 1 integrons were identified in 17 (19%) isolates from swine and 42 (47%) from chickens. The majority of integrons possessed genes conferring resistance to streptomycin and trimethoprim. These findings suggest that multiple-antimicrobial-resistant *E. coli* isolates, including fluoroquinolone-resistant variants, are commonly present among diseased swine and chickens in China, and they also suggest the need for the introduction of surveillance programs in China to monitor antimicrobial resistance in pathogenic bacteria that can be potentially transmitted to humans from food animals.**

Although normally commensal in nature, certain strains of *Escherichia coli* are associated with a variety of infections in humans and animals. In swine, pathogenic *E. coli* may cause neonatal and postweaning diarrhea and edema (6, 18). In chickens they may cause infections of the respiratory tract and soft tissues, resulting in colibacillosis, air sacculitis, and cellulitis (15). Virulence factors for swine *E. coli* include adhesins and several exotoxins (6, 12, 18). For example, fimbrial types F4 (K88), F5 (K99), F6 (987P), and F107 and intimin, an outer membrane protein encoded by the *eae* gene, play a role in adhesion to mucosal surfaces (18, 21, 28). Exotoxins produced by *E. coli* include heat-stable (STa and STb) and heat-labile (LT) enterotoxins, Shiga toxins (Stx1 and Stx2), and cytotoxic necrotizing factors (CNF1 and CNF2) (30, 35). In swine, the most commonly reported *E. coli* serogroups associated with neonatal and postweaning diarrhea and edema belong to a limited number of serogroups, including O8, O138, O139, O141, O147, and O157 (12, 13, 18, 25). Avian pathogenic *E. coli* most commonly belongs to O1, O2, or O78 and typically possesses virulence factors such as lipopolysaccharide, temperature-sensitive hemagglutination (Tsh), and increased serum survival factor (ISS) (21, 24, 35). However, the distribution and frequencies of the most prevalent serogroups can vary considerably, both geographically and temporally (12, 13).

Antimicrobials are valuable tools to treat clinical disease and to maintain healthy and productive animals. However, the treatment of whole herds and flocks with antimicrobials for disease prevention and growth promotion has become a controversial practice (34, 38). Sick animals are sometimes treated individually, but often whole flocks or herds of animals are treated at once, including animals that are not ill. In addition, antimicrobials are used in the absence of disease to prevent diseases during times when animals may be susceptible to infections. This practice is very common in China and other countries where infections caused by enteric pathogens are severe on poultry and swine farms. Such misuse and/or inappropriate usage affects a larger number of animals, because it again usually involves treating a whole herd or flock, which increases the likelihood of selecting for organisms that are resistant to the antibiotic. Of particular concern is the emergence of resistance to frontline antimicrobials, such as the fluoroquinolones, which because of their low toxicity and relatively broad-spectrum coverage are extremely valuable for treating human infections (2, 22). In addition to the human health concerns, antimicrobial-resistant pathogens also pose a

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severe and costly animal health problem in that they may prolong illness and decrease productivity through higher morbidity and mortality (39).

Unfortunately, data on the prevalence of antimicrobial-resistant veterinary pathogens are sparse, particularly in developing countries, including China, where antimicrobials are overused in veterinary medicine and food animals (4). Swine farms in China often experience neonatal diarrhea and diarrhea in young piglets with as high as 95% morbidity and 40% mortality. Colibacillosis on poultry farms is usually sporadic and a secondary infection from viral diseases. The morbidity and mortality are approximately 25% and 5%, respectively. Poultry farms often use ciprofloxacin, penicillin, and streptomycin to treat infected chicken flocks. Many fluoroquinolone drugs have been approved to be used in veterinary medicine (39), and it is a common practice in China that antimicrobials are used as feed supplements and that fermentation waste from antimicrobial production is used as food animal feed (4, 39). Data on the prevalence of antimicrobial-resistant veterinary pathogens are needed for science-based risk assessments focusing on the relative risks concerning use of antimicrobials in animal husbandry. The study presented here was undertaken to determine the serogroups, virulence factors, antimicrobial susceptibility profiles, presence of class 1 integrons, and molecular basis of fluoroquinolone resistance in *E. coli* organisms isolated from diseased swine and chickens from farms in China. The overall goal was to further our understanding of both pathogenic and antimicrobial resistance mechanisms present among *E. coli* in food-producing animals from an area which has not instituted or benefited yet from a national surveillance system.

MATERIALS AND METHODS

Bacterial isolates. A total of 160 *E. coli* isolates were analyzed in the study. These included 89 isolates recovered from fecal samples of 2- to 10-day-old piglets. The samples were recovered from animals experiencing diarrhea, located on three pig farms in Beijing, China, during August 2000. Seventy-one *E. coli* isolates were recovered from the livers of dead chickens from 10 poultry farms in Beijing and Heibei Province from January to October 2000. Antimicrobials including ciprofloxacin, penicillin, and streptomycin were used to treat the animals on the farms. All *E. coli* organisms were isolated and purified on MacConkey agar (Difco Laboratories, Detroit, Mich.) and confirmed as *E. coli* with the API20E bacterial identification system (bioMerieux, Inc., Hazelwood, Mo.).

Serogroup determination and identification of virulence genes. Serogroups and virulence genes were determined at the Gastroenteric Disease Center of Pennsylvania State University, State College. PCR was used to detect virulence genes, including those for LT, STa, STb, Stx1, Stx2, CNF1, CNF2, F4 (K88), F5 (K99), F6(987P), F18(F107), and intimin for swine *E. coli* and K1, ISS, Tsh, and HlyE for avian *E. coli*, using previously published primers and protocols (12, 24).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed according to National Committee for Clinical Laboratory Standards methods (26, 27). Antimicrobial MICs for the *E. coli* isolates were determined via broth microdilution using the PASCO MIC/ID system (Becton Dickinson, Cockeysville, Md.) according to the manufacturer's instructions. Antimicrobials included in the panels are listed in Table 1. In addition, MICs of nine quinolone antimicrobials (Table 1) were determined using the Sensititre automated antimicrobial susceptibility system (Trek Diagnostic Systems, Westlake, Ohio). *E. coli* strains ATCC 25922 and 35218, *Enterococcus faecalis* ATCC 51299, and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control organisms in all antimicrobial susceptibility tests.

Detection and sequence analysis of the QRDRs in *gyrA*, *gyrB*, *parC*, and *parE*. Chromosomal DNA was prepared using a Wizard genomic DNA purification kit (Promega, Madison, Wis.). The quinolone resistance-determining regions (QRDRs) of *gyrA*, *gyrB*, *parC*, and *parE* were amplified by PCR using previously published primers (Table 2) and protocols (11). The PCR was performed in a 50-μl volume consisting of a 0.25 mM concentration of each deoxyribonucleotide, 1.5 mM $MgCl_2$, 1 U of Gold *Taq* DNA polymerase, and 50 pmol of each primer. The temperature profile was 95°C for 10 min; 30 cycles of 95°C for 30 s, 55°C for 45 s, and 72°C for 45 s; and a final cycle of 72°C for 7 min.

Predicted polypeptide products were analyzed for amino acid changes by comparison with wild-type *E. coli gyrA* (accession number AE000312), *gyrB* (AE000447), *parC* (AE000384), and *parE* (AE000385).

Detection and sequence analysis of class 1 integrons. A PCR assay using 5′CS and 3′CS primers (Table 2) was used to identify class 1 integrons in the *E. coli* isolates, according to the method of Zhao et al. (40). Each of the amplified products was purified with a High-Pure PCR purification kit (Roche, Indianapolis, Ind.) and sequenced at the Center for Agriculture Biotechnology, University of Maryland, College Park. Resultant DNA sequence data were compared to data in the GenBank database via the BLAST algorithm (1).

RESULTS

Serogrouping and virulence genes. Six serogroups, consisting of O1, O25, O78, O84, O88, and O160, were identified among the 71 *E. coli* isolates from diseased chickens (Table 3). The majority of the chicken isolates (63%) were identified as serogroup O78. Seven isolates were nontypeable. *E. coli* isolates from chickens possessed multiple virulence factors (Table 3). Ninety-seven percent of isolates were PCR positive for the increased serum survival (*iss*) gene, and 93% were positive for the temperature-sensitive hemagglutinin (*tsh*) gene. None of the isolates contained the *hlyE* gene. Nine isolates carried all three virulence factors, K1, Iss, and Tsh, including four O25, two O78, two O88, and one nontypeable strain. Two isolates did not have any of the virulence factors examined.

In contrast to the avian *E. coli*, the O serogroups identified in swine *E. coli* were not those typically associated with pathogenic strains in the United States. Additionally, the swine isolates did not posses the characteristic toxin and fimbrial virulence factors associated with swine diarrhea (Table 3). Twenty-three serogroups were identified among the 89 *E. coli* isolates from swine; however, 38% were nontypeable. Among the 89 swine isolates, 10% contained Stx1, 7% contained CNF2, and 4 contained either STa, CNF2, K99, or F107. Two *E. coli* isolates possessed two virulence factors: O153 (K99 and CNF2) and O171 (Stx1 and CNF2). However, most (81%) isolates did not contain any of the virulence factors screened for in this study.

Antimicrobial resistance. Most *E. coli* isolates recovered from diseased chickens and swine were resistant to multiple classes of antimicrobials (Table 1). The majority of isolates were resistant to tetracycline (98%), sulfamethoxazole (84%), ampicillin (79%), streptomycin (77%), and trimethoprim-sulfamethoxazole (76%). Resistance to chloramphenicol among the swine isolates (63%) was significantly higher ($P < 0.05$) than resistance among chicken isolates (24%). Surprisingly, all *E. coli* isolates, regardless of their animal origin, were resistant to the quinolone antimicrobial nalidixic acid. Due to the high incidence of quinolone resistance, all isolates were also tested against a panel of veterinary-use and human-use fluoroquinolones. Among these isolates, fluoroquinolone resistance ranged between 64% to levofloxacin, 79% to ciprofloxacin, and 95% to difloxacin. *E. coli* isolates of both avian and swine origin displayed elevated levels of resistance to all fluoroquinolones tested (Table 1).

With regards to multidrug resistance profiles, all isolates from swine were resistant to more than 8 of the 19 antimicrobials tested, 86% were resistant to more than 11 antimicrobials, and 2% were resistant to 16 antimicrobials. All isolates recov-

TABLE 1. Antimicrobial resistance phenotypes of *E. coli* isolates from diseased chickens and piglets

Class and antimicrobial	Resistance breakpoint[a] (μg/ml)	Chicken isolates			Swine isolates			Overall % Resistance
		MIC_{50}[b] (μg/ml)	MIC_{90} (μg/ml)	% Resistance	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Resistance	
Cephalosporins								
Ceftiofur	8	≤0.5	1	0	≤0.5	≤0.5	0	0
Ceftriaxone	64	≤0.025	≤0.025	0	≤0.025	≤0.025	0	0
Cephalothin	32	8	>32	27	16	32	20	23
Penicillins								
Amoxicillin-clavulanic acid	32,16	8,4	8,4	0	8,4	16,8	0	0
Ampicillin	32	>32	>32	77	>32	>32	80	79
Sulfonamides and potentiated sulfonamides								
Sulfamethoxazole	512	>512	>512	79	>512	>512	89	84
Trimethoprim-sulfamethoxazole	4,76	>4,76	>4,76	63	>4,76	>4,76	87	76
Quinolones and fluoroquinolones								
Ciprofloxacin	4	>16	>16	73	16	>16	84	79
Difloxacin	4	>16	>16	91	>16	>16	98	95
Enrofloxacin	2	>16	>16	90	>16	>16	76	83
Gatifloxacin	8	16	>16	67	8	>16	75	72
Levofloxacin	8	16	>16	63	8	>16	65	64
Nalidixic acid	32	>256	>256	100	>256	>256	100	100
Orbifloxacin	8	>16	>16	76	>16	>16	96	87
Sarafloxacin	0.25	>16	>16	100	16	>16	100	100
Phenicols								
Chloramphenicol	32	>32	>32	24	4	>32	63	46
Aminoglycosides								
Gentamycin	16	0.5	>16	30	2	>16	29	29
Streptomycin	64	>256	>256	80	>256	>256	74	77
Tetracycline	16	>32	>32	100	>32	>32	96	98

[a] Values, with the exception of those for streptomycin (26, 27), are based on NCCLS standards.
[b] MIC_{50}, MIC at which 50% of isolates were inhibited.

ered from diseased chickens were resistant to at least 3 of the 19 antimicrobials tested. Fifty-six (79%) avian *E. coli* isolates were resistant to more than 8 antimicrobials, and two (3%) were resistant to 16 antimicrobials. All *E. coli* isolates from this study were susceptible to ceftiofur, ceftriaxone, and amoxicillin-clavulanic acid.

Topoisomerase point mutations in fluoroquinolone-resistant *E. coli*. All quinolone-resistant *E. coli* isolates were tested for amino acid substitutions in GyrA, and 39 representative isolates (20 from swine and 19 from chickens) were also tested for amino acid mutations in GyrB, ParC, and ParE (Table 4). All nalidixic acid-resistant *E. coli* isolates possessed a mutation at position 83 in GyrA (S83L). Single mutations (S83L) and double mutations (S83L and D87N, D87G, or D87Y) were found in GyrA, whereas no mutations in GyrB and only single mutations, S80I, S80R, or E84K in ParC and S459A or N463D in ParE, were identified. Among the 38 isolates that possessed mutations in ParC, the most common was S80I (90%), followed by S80R (8%). The most frequent pattern of mutations ($n = 18$) included a double mutation in GryA (S83L, D87N) and a single mutation in ParC (S80I). All 18 isolates exhibited resistance to both enrofloxacin and ciprofloxacin, and 94% were resistant to gatifloxacin. Less common mutations included four isolates with a mutation in GryA (S83L) and in ParC (S80I) and four isolates with a double mutation in GyrA (S83L, D87N) and single mutation in ParC (S80I) (Table 4). One isolate possessed only an S83L substitution in GyrA and was nalidixic acid resistant but susceptible to the fluoroquinolones tested, with the exception of sarafloxacin (MIC, 0.5 μg/ml).

TABLE 2. Primer sequences used to amplify class 1 integron and the QRDRs of *gyrA*, *gyrB*, *parC*, and *parE*

PCR target	Primers	Primer sequence (5′–3′)	Reference
Class 1 integron	5′CS	GGCATCAAGCACAAGC	40
	3′CS	AAGCAGACTTHACTGAT	
QRDR of *gyrA*	*gyrA*-1	ACGTACTAGGCAATGACTGG	11
	gyrA-2	AGAAGTCGCCGTCGATAGAA	
QRDR of *gyrB*	*gyrB*-1	TGTATGCGATGTCTGAACTG	11
	gyrB-2	CTCAATAGCAGCTCGGAATA	
QRDR of *parC*	*parC*-1	CAGACTGCCAGGACGCGAT	11
	parC-2	AGCCAAGCGCGGTGATAAGC	
QRDR of *parE*	*parE*-1	TACCGAGCTGTTCCTTGTGG	11
	parE-2	GGCAATGTGCAGACCATCAG	

Presence of class 1 integrons. All veterinary *E. coli* isolates were characterized for the presence of class 1 integrons, since a recent study documented plasmid-mediated quinolone resistance in clinical *E. coli* isolates from Shanghai, China, and suggested the possibility that the gene, *qnr*, is possibly located

TABLE 3. Serogroups and virulence genes of *E. coli* isolates from diseased chickens and swine[a]

E. coli isolate source and O group(s)	No. of isolates	No. with virulence factor									
		K1	ISS	Tsh	HlyE	STa	Stx1	CNF1	CNF2	K99	F107
Chickens											
O1	2	0	1	1	0						
O25	4	4	4	4	0						
O78	45	2	45	45	0						
O84	6	0	6	6	0						
O88	6	2	6	3	0						
O160	1	0	0	0	0						
NT	7	1	7	7	0						
Total	71	9	69	66	0						
Swine											
O1						0	1	0	0	0	0
O21						0	0	1	0	0	0
O101						0	1	0	1	1 (O153)	0
O9, O88, O153 and O171						0	1 (O9)	0	1 (O153), 1 (O171)	0	0
O19, O25, O91, O142, O149, O157, and O159						0	2 (O91)	0	1 (O159)	0	1 (O149)
O33, O35, O54, O79, O114, O160, X3, X13, and X28						1 (O114)	1 (X13)	0	0	0	0
NT						0	3	0	2	0	0
Total						1	9	1	6	1	1

[a] K1, capsule antigen K1; ISS, increased serum survival factor; Tsh, temperature-sensitive hemagglutination; HlyE, hemolysin E; STa, heat-stable enterotoxins; Stx1, Shiga toxin 1; CNF1 and CNF2, cytotoxic necrotizing factors 1 and 2; K99, fimbrial type 5; F107, fimbrial type 107; X, experimental O group; NT, nontypeable.

in a class 1 integron (37). Nineteen percent of *E. coli* isolates recovered from swine possessed class 1 integrons, with sizes ranging from ca. 0.7 to 2.0 kb. Nine different serotypes of swine *E. coli* isolates harbored integrons; however, the majority of serotypes harboring these integrons were nontypeable. Ten isolates carried a 1.5-kb integron containing the *dhfr1* and *aadA1* genes (Table 5), which confer resistance to trimethoprim and streptomycin, respectively. Three isolates carried a 2.0-kb integron. In two isolates, the 2.0-kb integron contained *dhfr12* and *aadA2*, and in one strain it contained *dhfr17* and *aadA2*. Two isolates contained a 1.0-kb integron (*aadA1*), with one strain containing an additional 1.5-kb integron (*dhfr1* and *aadA1*). The majority (59%) of avian *E. coli* isolates contained class 1 integrons ranging in size from 0.7 to 1.5 kb (Table 5). Integrons were found in avian isolates from four of the six serogroups identified; however, the majority were found within isolates possessing serogroup O78 (35 of 45). Thirty-four of these isolates contained a 1.5-kb integron with *dhfr1* and *aadA1*, and one isolate contained a 1.5-kb integron with *dhfr17* and *aadA2*. Six isolates contained both the 1.5-kb integron (*dhfr1 and aadA1*) and the 0.7-kb integron (*dhfr13*). Two isolates contained the 1.0-kb integron with the *aadA1* gene. No class 1 integrons were found that possessed the plasmid-mediated quinolone resistance gene, *qnr*.

DISCUSSION

More than 160 O serogroups have been identified in *E. coli*. However, disease-causing *E. coli* typically consists of a rela-

TABLE 4. Amino acid substitutions in DNA gyrase and topoisomerase IV and corresponding fluoroquinolone resistance profiles among *E. coli* isolates from diseased chickens and swine ($n = 39$)

No. of isolates	Amino acid substitution(s)[a]			MIC range (μg/ml)			
	GyrA	ParC	ParE	Nalidixic acid	Enrofloxacin	Ciprofloxacin	Gatifloxacin
1	S83L			256	0.5	0.25	0.25
3	S83L	S80R		>256	2–8	1–4	1–4
4	S83L	S80I		>256	1–4	0.5–4	0.5–2
1	S83L D87N	S80I		>256	16	8	4
1	S83L D87G	S80I		>256	16	16	8
1	S83L D87N	E84K		>256	>16	16	8
23	S83L D87N	S80I		>256	16–>16	8–>16	4–>16
2	S83L D87N	S80I	S459A	>256	16–>16	8–>16	8–>16
1	S83L D87Y	S80I		>256	16–>16	8–>16	8–>16
1	S83L D87Y	S80I	S459A	>256	16–>16	8–>16	8–>16
1	S83L D87Y	S80I	N463D	>256	16–>16	8–>16	8–>16

[a] Substituted amino acid(s) and the position number are shown, e.g., S83L indicates substitution of a leucine for a serine at position 83 Amino acids: S, serine; L, leucine; D, aspartic acid; N, asparagine; Y, tyrosine; I, isoleucine; R, arginine; G, glycine; A, alanine.

TABLE 5. Integrons and their gene cassettes in swine and avian *E. coli* isolates

Size of integron (kb)	No. of strains		Gene cassette[a]
	Swine (n = 89)	Avian (n = 71)	
2.0	2	0	*dhfr12 aadA2*
2.0	1	0	*dhfr17 aadA2*
1.5	10	33	*dhfr1 aadA1*
1.5	0	1	*dhfr17 aadA2*
1.0	3	3	*aadA1*
0.7	0	1	*dhfr13*
1.5, 1.0	1	0	*dhfr17 aadA2*, *aadA1*
1.5, 0.7	0	4	*dhfr17 aadA2*, *dhfr13*

[a] *aad*, aminoglycoside adenyltransferase; *dhfr*, dihydrofolate reductase.

tively few serogroups. In this study, 66% of the *E. coli* isolates from chickens belonged to serogroups (O1 and O78) typically associated with colibacillosis. The ability of these isolates to cause disease in the birds may have been attributable, in part, to the fact that they possessed ISS and TSH, virulence factors known to increase bacterial resistance to serum and colonization of internal organs of infected chickens (21, 24). The finding that most isolates were O78 might be useful to poultry farms in combating *E. coli* infections and offer an alternative to antibiotics in the form of bacteria vaccines. In contrast to those from chickens, *E. coli* isolates from diseased piglets were found to have O serogroups different from their counterparts in other regions of the world. These isolates did not possess the virulence factors seen in serogroups such as O8, O147, O149, and O157, which are often associated with diarrhea in piglets (30). Thus, while it is possible that other colonization factors or enterotoxins may have contributed to the pathogenicity of these *E. coli* isolates, they might just be generic *E. coli* or the infections might have been caused by viruses. Misuse of antimicrobials in viral infections can contribute to the emergence of antimicrobial resistance in bacteria as well.

Similar to the findings of previous studies (5, 29, 31), most *E. coli* isolates of avian and swine origin described here were resistant to tetracyclines, aminoglycosides, and sulfonamides. Many swine *E. coli* isolates (64%) were resistant to chloramphenicol, which was also observed in 53% of swine *E. coli* isolated from Oklahoma farms, although the drug has been prohibited for use in food animals in the United States since the mid 1980s (7). One possibility for the prevalence of the chloramphenicol resistance phenotype in the United States is that a plasmid carrying the *cmlA* gene is widely disseminated and chloramphenicol resistance may be coselected with other antimicrobials. Chloramphenicol, however, is still widely used in swine in China (4). Perhaps the most striking finding from this study was the widespread resistance to quinolones and fluoroquinolones. Consequently, fluoroquinolones have become ineffective in veterinary medicine in China (39). All *E. coli* isolates were resistant to nalidixic acid and sarafloxacin. More than 70% were resistant to ciprofloxacin, and more than 60% were resistant to the newer human fluoroquinolones, gatifloxacin and levofloxacin. Somewhat similar findings have been reported in a recent study of clinical *E. coli* isolates from China, wherein greater than 50% of all isolates were resistant to ciprofloxacin (36). Similar findings were also reported for *E. coli* isolates recovered from chickens and swine in Spain, where 90% of chicken isolates and 50% of swine isolates were resistant to ciprofloxacin (29). Fluoroquinolone resistance in these isolates, coupled with the observation of widespread multiple antimicrobial resistance (e.g., 80% of the isolates from this study were resistant to eight or more antibiotics), likely portends further complications in treatment of *E. coli* infections in humans and animals from this region.

The molecular investigations into the underlying quinolone resistance mechanisms revealed that all quinolone-resistant isolates possessed the typical mutations in the topoisomerase genes, *gyrA* and *parC*, reported by other studies (3, 17). With regards to correlation of mutations with decreased susceptibilities to tested fluoroquinolone agents, isolates with single mutations in GyrA had relatively low ($\leq$4 μg/ml) MICs of enrofloxacin and ciprofloxacin (Table 4), whereas many *E. coli* isolates with double GyrA and single ParC mutations had correspondingly high MICs ($\geq$8 μg/ml) of these drugs. Among the 38 isolates that possessed ParC mutations, almost all possessed a mutation at position 80 resulting in an amino acid substitution of either arginine (S80R; n = 3) or isoleucine (S80I; n = 34), with the exception of one swine isolate, which carried the E84K mutation in *parC*. Mutations in *parC* at S80I or E84K have been associated with high-level resistance to fluoroquinolones and have been detected in clinical strains carrying a *gyrA* mutation (3, 17, 20). As expected, single mutations observed in *parC* always coincided with mutations in *gyrA* among the veterinary *E. coli* isolates in this study. Mutations in *gyrB* and *parE* have been associated with quinolone resistance (16); however, the mutation frequency is much lower compared to those for *gyrA* and *parC*. Additionally, the *parE* mutations reported here have to the best of our knowledge not been reported previously (8, 19). However, it is difficult to determine how much these mutations contribute to decreased fluoroquinolone susceptibility, as all isolates that possessed *parE* mutations also carried mutations in *gyrA* and *parC*. Other mechanisms of fluoroquinolone resistance exist besides mutations in the genes encoding DNA gyrase and topoisomerase IV. These include decreased production of porin proteins and up-regulation of multidrug resistance efflux pumps (14). Though we did not explore the contribution of these other mechanisms to the fluoroquinolone-resistant phenotypes observed in this study, it is more than likely that they have played a role in the evolution of fluoroquinolone resistance. The isolates in this study are the subject of future research to determine if efflux mechanisms are also involved in the quinolone-resistant phenotypes.

Regarding the genetics of resistance to other antimicrobials in the *E. coli* isolates from this study, integrons were likely important, both in terms of the mechanisms of resistance and in the dissemination of resistance genes (9, 33). Integrons are known to be associated with multiple-drug resistance in enteric organisms, and class 1 integrons specifically have been shown to be important in the dissemination of *intI*, *sulI*, and one or more antimicrobial resistance gene cassettes among gram-negative bacteria (23, 40). The findings from this study are consistent with most previous data, in that varying sizes of integrons were found in the *E. coli* isolates and they contained genes for resistance to aminoglycosides (5), trimethoprim, and β-lactams (10, 32, 40). More recently, Wang et al. (37) re-

ported the occurrence of a gene in a class I integron, termed *qnr*, which provides low-level quinolone resistance. They found *qnr* in fluoroquinolone-resistant human clinical *E. coli* isolates from Shanghai, China, suggesting the possibility that *qnr* was also present in some of our avian and swine strains. None of the integron-positive avian or swine *E. coli* isolates yielded *qnr* (data not shown) or a *qnr*-like gene upon DNA sequencing; however, further studies are ongoing on these isolates to determine if *qnr* is indeed present, but not in the class 1 integrons previously identified.

In summary, because trained practitioners are unavailable in many regions of developing countries, regulations on the veterinary use of antibiotics are poorly enforced or absent. Consequently, there is opportunity for the inappropriate use of antibiotics in both human and veterinary medicine. With concentrated livestock production increasing in developing countries, reliance on antimicrobials will likewise expand. Our data suggest that a lack of restrictions on antimicrobial use in food animals in China has resulted in the dissemination of multiple-drug-resistant pathogenic *E. coli* isolates, including fluoroquinolone-resistant variants. Research is needed to determine the role of antimicrobial use in animal production environments in the emergence and spread of resistance in both veterinary and human medicine worldwide, especially in developing countries. It is also important that antimicrobial prudent-use guidelines be developed in conjunction with the establishment of surveillance programs in China, similar to the National Antimicrobial Resistance Monitoring System in the United States, for monitoring antimicrobial resistance in pathogenic bacteria that can be transmitted to humans from food animals. This information should ultimately provide important guidance for the development of public health policy for use of antimicrobials in food animal production in developing countries.

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